Purpose: There is increasing evidence that the fragments of fibronectin mediate the breakdown of articular cartilage and are potentially the cause of the early damaging effects in osteoarthritis (OA). In particular, the competing mechanisms for the catabolic and anabolic factors, driven respectively by fibronectin fragments (FN-fs) and mechanical loading will influence the balance of these pathways. The present study examined the effects of FN-fs on catabolic activities in chondrocyte/agarose constructs subjected to dynamic compression under different oxygen tension.

Methods: Chondrocyte/agarose constructs were cultured under fluid-flow, osmotic or static conditions or subjected to dynamic compression (15 %, 1 Hz) with 0 or 1 μM amino-terminal Fn-f (29 kDa) and/or 1 mM L-NIO (inhibits NOS) at 1, 5 and 21 % oxygen tension (v/v) for up to 48 hr. In addition, constructs were cultured with 0 or 10 ng / ml IL-1β and/or L-NIO to compare the effects of the cytokine with FN-fs. NO production, MMP activity and expression of iNOS and COX-2 were analysed using biochemical assays. MMP activity was analysed using a fluorogenic substrate assay. Real-time qPCR assays coupled with molecular beacons were used to quantify gene expression of catabolic (iNOS, COX-2) and anabolic (aggrecan, collagen type II) signals by normalizing each target to GAPDH. 2-way ANOVA and a post hoc Bonferroni-corrected t-test were used to analyse the data.

Results: Both FN-fs and IL-1β significantly increased the levels of NO, PGE2 and MMP activity (p<0.001) in constructs cultured under 21 % oxygen (Fig. 1). The catabolic response was significantly enhanced in FN-f treated constructs cultured under 5 % oxygen tension and the response was reduced in the presence of the NOS inhibitor (all p<0.001). In addition, the presence of FN-fs but not IL-1β significantly inhibits GAG synthesis at 5 (p<0.001) or 21 % oxygen tension (p<0.001) in constructs cultured for 48 hr. In unstrained constructs, FN-fs or IL-1β increased the levels of NO, PGE2 and MMP activity and expression of iNOS and COX-2 in an oxygen dependent manner with maximal levels at 5 % (all p<0.001). The application of dynamic compression reduced catabolic activities and the response was further reduced with L-NIO. Dynamic compression increased GAG synthesis (p<0.001) and gene expression of aggrecan and collagen type II and the response was abolished FN-f induced catabolic response. Interestingly, low oxygen tension (5 %) exacerbated FN-f induced catabolic activities, but did not affect the loading-induced recovery. These findings indicate that FN-fs exert catabolic effects in an oxygen dependent manner and the response was prevented with biomechanical signals. The combination of mechanical and pharmacological interventions with NOS inhibitors makes this study a useful tool to examine further the interactions of biomechanics and cell signalling in OA.

Conclusions: The present findings demonstrate that FN-fs stimulate catabolic activities via an iNOS dependent pathway, resulting in NO production, MMP activity, and PGE2 release. The effect of FN-f was more potent than the cytokine and the response was dependent on oxygen tension. In addition, stimulation with biomechanical signals reduced catabolic activities and co-stimulation with the NOS inhibitor abolished FN-f induced catabolic response. Interestingly, low oxygen tension (5 %) exacerbated FN-f-induced catabolic activities, but did not affect the loading-induced recovery. These findings indicate that FN-fs exert catabolic effects in an oxygen dependent manner and the response was prevented with biomechanical signals. The combination of mechanical and pharmacological interventions with NOS inhibitors makes this study a useful tool to examine further the interactions of biomechanics and cell signalling in OA.
understood. Chondrocytes are directly responsible for cartilage remodeling and are able to regulate their metabolic activities based on surrounding mechanical signals. In this study, we hypothesize that exposure to ZA can directly change the mechanosensation behaviors of chondrocytes. Our aim is to investigate the fluid flow induced intracellular calcium signaling of chondrocytes with ZA treatment.

Methods: 1) Chondrocytes: Hyaline cartilage was harvested from condyle head in calf knee joints. Tissue was then digested in 2.5mg/ml pronase and 0.5mg/ml of collagenease at 37 C. Isolated cells were cultured in DMEM with 10% FBS and 1% P/S for three days before testing. Cells were suspended and seeded on glass slides (150,000 cells/slide) 24 hours before calcium imaging.
2) Fluid Flow and Calcium Imaging: Cells were dyed with Fluo-4 AM (Fig. 2A), and then glass slide was mounted in a laminar flow chamber. Fluid flow was controlled by a magnetic pump to apply 2 Pa shear stress on the cell surface. Fluorescent images of cells were recorded with a high speed EMCCD camera for 10 minutes (1 min for baseline and 9 minutes after the onset of flow). Intracellular calcium intensity fluctuations of each individual cell were obtained by imaging analysis. A set of spatiotemporal parameters were defined and compared to evaluate the calcium responses of cells between different groups.
3) Experimental Groups: (i) Untreated; (ii) 1µM ZA, cells were incubated in 1µM ZA media before and during flow study; (iii) 10 µM ZA, cells were treated with 10 µM ZA.

Results: Under the stimulation of fluid flow, primary chondrocytes demonstrated strong intracellular Ca2+ responses (Fig 3). A large portion of the cells released one or more calcium peaks in 9 minutes. The percentage of responsive cells was significantly higher in the untreated group (33.4%) than that of 1 µM ZA group (32.4%) and 10 µM ZA (22.1%) (Fig. 3E). For responsive cells, there were no differences in the number of peaks between three groups (Fig. 3A). Magnitude of calcium peaks in untreated group was significantly higher than those in ZA treated groups (Fig. 3B). Each cell responded at different time points after the onset of flow, and no differences were detected between the experimental groups for the response time of the first calcium peaks (Fig. 3C). Cells in 10 µM ZA group relaxed faster from the calcium peak than those in the other two groups.

Conclusions: As one of the earliest responses of chondrocytes under mechanical stimulation, calcium signaling is the upstream of numerous other mechanotransduction pathways. We found that ZA treatment can significantly affect the calcium signaling of primary chondrocytes, especially the percentage of responsive cells under fluid flow stimulation. High concentration of ZA reduced the mechano-sensitivity of chondrocytes to mechanical stimulation. Interestingly, the number of calcium peaks of the responded cells has no difference between three groups. Therefore, effect of ZA on chondrocyte calcium signaling has an "all or nothing" character. Our results provided new knowledge about the chondro-protective function of ZA in PTOA.

Figure 1. Continuous administration of the FDA approved drug zolendronic acid(ZA), a bisphosphonate to treat bone loss, suppressed the development of post-traumatic OA in a DMM mouse model (destabilization of the medial meniscus). A) Histological analysis and B) Cartilage Damage Scoring of DMM and ZA treated mouse knee. (Veh: Vehicle control group).

Figure 2A. Fluo-4 stained Fluorescent image of chondrocytes Seeded on a glass slide. 2B. Schematic of fluid flow chamber. 2C. Typical calcium response curve over time.

Figure 3. Analysis on Calcium response fluctuation.

402 EFFECT OF MECHANICAL STRESS ON THE HYPERTROPHIC DIFFERENTIATION RELATED GENE EXPRESSION IN CULTURED CHONDROCYTES

H. Inoue, Y. Arai, R. Terauchi, S. Nakagawa, M. Saito, N. Hirooka, S. Tsuichida, T. Matsuui, O. Mazda, T. Kubo, Dept. of Orthopaedics, Graduate Sch. of Med. Sci., Kyoto Prefectural Univ. of Med., Kyoto, Japan; Dept. of Rheumatoid Diseases and Joint Function, Kyoto Prefectural Univ. of Med., Kyoto, Japan; Dept. of Immunology, Kyoto Prefectural Univ. of Med., Kyoto, Japan.

Purpose: In vivo chondrocytes were exposed to various types of mechanical stress such as hydrostatic pressure, shear stress and compressive stress. Among these, it is considered that hydrostatic pressure is most closely involved in the pathogenesis of OA. Recently, many hypertrophic differentiation related factors including matrix metalloproteinase (MMP), and NF-kB/hypoxia inducible factor (HIF) -2x signal have been reported to play an important role in initiation or progression of osteoarthritis (OA). However, the influence of hydrostatic pressure on these molecules expression is not clarified.

In light of the above, we analyzed the relation between hydrostatic pressure and HIF-2x, MMP-3 and MMP-13.

Methods: Chondrocytes were isolated from male 1.2-1.5 kg Japanese white rabbits. The isolated chondrocytes were cultured as monolayers in Petri dishes. Chondrocytes in Petri dishes were exposed to continuous hydrostatic pressure of 5 or 50MPa for 2 hours. The Petri dishes were placed in a Teflon pouch and the pouch then was placed in a stainless-steel pressurization vessel, equipped with an oil pressure apparatus. The temperature was maintained at 37 C. After depressurization, each of the Petri dishes was given 2 ml of fresh medium and was maintained at atmospheric pressure in the 5% CO2 environment until analysis. Cells seeded in Petri dishes and placed in the same apparatus under the same conditions were used as nonpressurized controls.

Thirty minutes after depressurization, total RNA was extracted and extracted RNAs were reverse transcribed. And gene expression levels of HIF-2x, MMP-3 and MMP-13 were measured by quantitative real-time PCR. Moreover, HSP70 gene expression was used as a marker for evaluation of hydrostatic pressure exposed to cultured chondrocytes.

Results: Continuous hydrostatic pressure increased gene expression of HSP70 in a pressure dependent manner. Although gene expression of HIF-2x was increased significantly when the cells were exposed to 5MPa of hydrostatic pressure compared with control group, that was decreased to the same level as control group when the hydrostatic pressure rose up to 50MPa (Figure). And gene expression change of MMP-3 and MMP-13 was similar to that of HIF-2x.

Conclusions: HIF-2x gene expression was changed in response to hydrostatic pressure exposed to cultured chondrocytes. Recent studies have shown that HIF-2x induces gene expression of MMP-3 and MMP-13. Our results indicated that gene expression of MMPs in cultured chondrocytes when they were loaded hydrostatic pressure varies similarly as the change of HIF-2x gene expression. Hypertrophic differentiation of chondrocytes induced by hydrostatic pressure may enhance the expression of MMP-3 or MMP-13 via HIF-2x.